Data Evaluation Record on the aerobic biotransformation of 1,2-benzisothiazolin-3-one (BIT) in soil

PMRA Submission Number {.....} EPA MRID Numbers 41199102 and 41199103

Data Requirement: PMRA Data Code:
EPA DP Barcode:
OECD Data Point:
EPA Guideline: 162-1

Test material:
Common name: 1,2-Benzisothiazolin-3-one.
Chemical name:
IUPAC name:
CAS name:
CAS No: 2634-66-5.
Synonyms: BIT.
SMILES string:

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Company Code:
Active Code:
Use Site Category:
EPA PC Code: 098901

CITATIONs:
MRID 41199102: Roberts, G. C. 1989. 1,2 Benzisothiazolin-3-one: aerobic degradation in soil. Unpublished study performed by Imperial Chemical Industries PLC, Brixham, Devon, United Kingdom at ICI Agrochemicals, Bracknell, Berkshire, United Kingdom; sponsored and submitted by Specialty Chemicals, ICI Americas Inc., Wilmington, DE. Study No.: R048/D (p. 3). Report No.: BL/B/3496. Experiment started May 2, 1988 and completed April 19, 1989 (p. 6). Final report issued April 19, 1989.

MRID 41199103: Powell, B. 1989. 1,2-Benzisothiazolin-3-one (BIT): identification of metabolites formed by aerobic degradation in soil, Report No. D97198B. Unpublished study performed by Imperial Chemical Industries Limited, Blackley, Manchester, United Kingdom; sponsored and submitted by Specialty Chemicals, ICI Americas Inc., Wilmington, DE. Report No.: D97198B. Experiment started September 2, 1988 and completed March 1, 1989 (p. 8). Final report issued June 12, 1989.

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EXECUTIVE SUMMARY

The aerobic soil metabolism half data requirement is satisfied for BIT with MRIDs 41199102 and 41199103. The half-lives ranged from 5.6-12.2 days for the anticipated application rate (3 mg/kg) and 16-17 days for the exaggerated rate (100 mg/kg) that was intended to generate any degradates that may form. The observed half-lives were 1-7 days for the 3 mg/kg rate. There were no significant degradates that occurred on a consistent basis, although two did show sporadically. These were ortho-sulphobenzamide and BIT-S-oxide.

The aerobic biotransformations of [^{14}C-ring]- and [^{14}C-carbonyl]-labeled 1,2-benzisothiazolin-3-one (BIT; radiochemical purities 91.7% and 94.9%, respectively) were studied in a sandy loam soil (pH 6.5, organic carbon 1.5%) from England for up to 180 days at 20°C and a moisture content of 40% of maximum water holding capacity (MWHC value at zero suction; darkness was not specified). Radiolabeled BIT was applied at a rate of 3 mg a.i./kg, equivalent to 0.5 lb/acre, the predicted annual application rate. The test materials were also applied at 100 mg a.i./kg for the metabolite identification rate. The experiment was conducted in accordance with USEPA Subdivision N Guideline §162-1 and in compliance with general, unspecified GLP standards. The test system consisted of glass pots (38 mm diameter) containing moist treated soil (20 g dry wt) that were loaded into glass columns using stainless steel wire racks. Humidified, carbon dioxide-free air was passed through the column. Volatiles in the out-going air were trapped using tubes filled with the following sequential series of sorbents: glass wool; 0.1M HCl; 2-methoxyethanol; ethanolamine; ethanolamine; glass wool; 2M NaOH; and 0.05M H_2SO_4. Duplicate samples of the 3 mg/kg treatment were collected at time 0 and 1, 4, 7, 14, 28, 60, 98, 125, and 180 days posttreatment. For the 100 mg/kg treatment, duplicate samples were collected at time 0 and 7, 14, 28, 98 and 180 days posttreatment. Soil samples were extracted on a cellulose thimble in a Soxhlet extraction apparatus with acetonitrile then acetonitrile:1M HCl (80:20, v:v). Soil residues were analyzed for total radioactivity using LSC/combustion. Volatiles were measured via LSC. The radioactivity in the NaOH traps was characterized as carbon dioxide using $BaCl_2$ precipitation prior to LSC analysis. Soil extracts were analyzed for the parent using normal- and reversed-phase TLC analysis. The parent, BIT, was identified via co-chromatography with the unlabeled BIT reference sample. In a subsequent analysis (MRID 41199103), further confirmation of the identification of the parent was performed with the selected high-dose (100 mg/kg) extracts which contained >10% of the applied radioactivity (day 7 ring-labeled and day 28 both labels) using TLC analyses. TLC, HPLC and HPLC/MS analyses were performed for identification of the transformation products. The following reference standards were included for identification of the transformation products: BIT-S-oxide (1,2-benzisothiazolin-3-one-1-oxide); saccharin (1,2-benzisothiazolin-3-one-1,1-dioxide); and o-sulphobenzamide (2-carbamoylbenzenesulphonic acid).

For the **3 mg/kg dose** rate on East Jubilee sandy loam soil, the overall recovery was 99.13 ± 1.82% (mean, range 94.74-102.41%) of the applied radioactivity for the [^{14}C-ring]BIT and 91.67 ± 10.27% (mean, range 81.96-103.06% with one outlier at time 0 of 69.94%) for the [^{14}C-carbonyl]BIT. There was a noticeable pattern of decline in the [^{14}C-carbonyl]BIT treatment. For the [^{14}C-ring]BIT treatment, the total extractable residues declined rapidly from the maximum of 88.96% of the applied radioactivity at time 0 to 41.86% at 1 day to 19.61-29.86% at 7-28 days to 6.13% at 60

days and was 2.60% at 180 days (study termination). Corresponding nonextractable residues increased from 11.82% at time 0 to a maximum of 72.98% at 7 days then declined to 54.29-54.73% at 125-180 days. For the [^{14}C-carbonyl]BIT treatment, the total extractable residues declined rapidly from the maximum of 67.80% at time 0 to 38.44% at 1 day to 10.59% at 28 days to 4.09% at 60 days and was 2.34% at 180 days (study termination). Corresponding nonextractable residues increased from 18.17% at time 0 to a maximum of 70.60% at 28 days then declined to 42.99% at 180 days. At 180 days, $^{14}CO_2$ was measured at maximums of 42.95% and 37.12% for the [^{14}C-ring]BIT and [^{14}C-carbonyl]BIT, respectively, applied to East Jubilee sandy loam soil at 3 mg/kg. For the **100 mg/kg dose** rate on East Jubilee sandy loam soil, the overall recovery was 95.99 ± 3.46% (mean, range 91.36-102.73%) for the [^{14}C-ring]BIT and 97.47 ± 3.07% (mean, range 91.92-101.61%) for the [^{14}C-carbonyl]BIT. There was a noticeable pattern of decline for both experiments. For the [^{14}C-ring]BIT, the total extractable residues declined rapidly from the maximum of 96.47% of the applied radioactivity at time 0 to 51.10% at 7 days to 37.07-43.63% at 14-28 days to 6.70% at 98 days and was 3.78% at 180 days (study termination). Corresponding nonextractable residues increased from 3.09% at time 0 to a maximum of 58.47% at 14 days then declined to 44.00% at 180 days. For the [^{14}C-carbonyl]BIT treatment, the total extractable residues declined rapidly from the maximum of 94.25% at time 0 to 53.80% at 7 days to 37.24-39.97% at 14-28 days to 3.38% at 98 days and was 2.90% at 180 days (study termination). Corresponding nonextractable residues increased from 5.03% at time 0 to a maximum of 54.87% at 14 days then declined to 34.65% at 180 days. $^{14}CO_2$ was measured at maximums of 43.81% (180 days) for the [^{14}C-ring]BIT and 55.62% (98 days; 55.07% at 180 days) for the [^{14}C-carbonyl]BIT. Other organic volatiles were observed in insignificant quantities in both experiments.

The amounts of the parent, 1,2-benzisothiazolin-3-one (BIT), were not determined throughout the study (MRID 41199102) or the supporting study (MRID 41199103). The study author reported that "only trace amounts" of the parent were present in the soil extracts sampled at 1 and 4 days posttreatment. The half-life of the parent, BIT, could not be determined since the observed amounts were not quantified in the study. However, the study author of MRID 41199102 reported that the aerobic degradation half-life of BIT was estimated to be "less than one day" when applied to sandy loam soil at 3 mg/kg and maintained at 20°C. The study author's estimation was based on the fact that the TLC plates of soil extracts at 24 hours posttreatment showed only a trace of BIT.

Transformation products were not determined in the initial study (MRID 41199102); however, selected high dose samples were analyzed for transformation products in a follow-up study (MRID 41199103). o-Sulphobenzamide (2-carbamoylbenzenesulphonic acid) was observed as a major transformation product in all samples (day 7 ring-labeled and day 28 both labels) with maximums of 26.47% and 28.46% at 28 days for the [^{14}C-ring]BIT and [^{14}C-carbonyl]BIT, respectively (14.16% at 7 days for the [^{14}C-ring]BIT. BIT-S-oxide (1,2-benzisothiazolin-3-one-1-oxide) was observed as a major transformation product in the [^{14}C-ring]BIT samples at 7 days (23.79%) and a minor transformation product at 28 days (1.89% and 1.72% for the [^{14}C-ring]BIT and [^{14}C-carbonyl]BIT, respectively). Saccharin (1,2-benzisothiazolin-3-one-1,1-dioxide) was observed as a minor transformation product in all samples with maximums of 5.94% at 7 days for the [^{14}C-ring]BIT and 4.32% at 28 days for the [^{14}C-carbonyl]BIT. Unknown radioactivity was measured at maximums of 1.24% and 1.73% at 28 days for the [^{14}C-ring]BIT and [^{14}C-carbonyl]BIT, respectively.

The study author of MRID 41199103 described a transformation pathway for 1,2-benzisothiazolin-3-one (BIT). BIT-S-oxide (1,2-benzisothiazolin-3-one-1-oxide) and saccharin (1,2-benzisothiazolin-3-one-1,1-dioxide) were formed by the sequential oxidation of the parent, BIT. o-Sulphobenzamide (2-carbamoylbenzenesulphonic acid) was formed by ring hydrolysis of saccharin. Extensive mineralization to carbon dioxide and formation of bound residues were observed.

Results Synopsis: (for 3 mg/kg rate)

Linear half-life: 38-42 days (first order regression).
Nonlinear half-life: 5.6-12.2 days (based on first order analysis)
Observed DT50: 1-7 days (based on linear interpolation)

Major transformation products for both labels:
- o-Sulphobenzamide (2-carbamoylbenzenesulphonic acid).
- BIT-S-oxide (1,2-benzisothiazolin-3-one-1-oxide, ring label).
- Carbon dioxide.

Minor transformation product for both labels:
- Saccharin (1,2-benzisothiazolin-3-one-1,1-dioxide).
- BIT-S-oxide (1,2-benzisothiazolin-3-one-1-oxide; carbonyl label).

Study Acceptability: This study is classified as acceptable and satisfies the 162-1 data requirement for BIT.

I. MATERIALS AND METHODS

All page numbers were based on the lower right-hand corner of the pages of the studies. The MRID from which the information was taken is included in the citation.

GUIDELINE FOLLOWED: Both studies were conducted in accordance with USEPA Pesticide Assessment Guidelines Subdivision N, § 162-1 (pp. 1, 6 of MRID 41199102; p. 1 of MRID 41199103).

COMPLIANCE: This study was conducted in compliance with principles of GLP (the origin of the GLP principles was not reported in either study; p. 3 of MRID 41199102; p. 3 of MRID 41199103). Signed and dated Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-3 of MRID 41199102; pp. 2-3, 5 of MRID 41199103). Certificate of Authenticity statements were not provided.

A. MATERIALS:

1. Test Material:		1,2-Benzisothiazolin-3-one (BIT; p. 7; Figure 1, p. 24 of MRID 41199102).
Chemical Structure:		See DER Attachment 1.
[^{14}C-Ring]BIT		
Description:		
Purity:	Radiochemical purity:	91.7% (by TLC; p. 7; Table 3, p. 17 of MRID 41199102).
	Batch/Reference No.	88-21.
	Analytical purity:	Not reported.
	Specific activity:	5.15 KBq/µg.
	Location of the radiolabel:	Uniformly in the benzyl ring (Figure 1, p. 24 of MRID 41199102).
Storage conditions of test chemical:		Not reported.
[^{14}C-Carbonyl]BIT		
Description:		
Purity:	Radiochemical purity:	94.9% (by TLC; p. 7; Table 3, p. 17 of MRID 41199102).
	Batch/Reference No.	88-35.
	Analytical purity:	Not reported.
	Specific activity:	0.74 KBq/µg.
	Location of the radiolabel:	On the carbonyl carbon (Figure 1, p. 24 of MRID 41199102).
Storage conditions of test chemical:		Not reported.

Physico-chemical properties of 1,2-benzisothiazolin-3-one:

Parameter	Value	Comment
Molecular weight	Not reported.	
Water Solubility (mg/L)	Not reported.	
Vapor Pressure (mmHg)	Not reported.	
UV Absorption	Not reported.	
pKa	Not reported.	
log P_{ow}	Not reported.	
Stability of compound at room temperature	Not reported.	

2. Soil Characteristics

Table 1: Description of soil collection and storage.

Description	East Jubilee
Geographic location	Jealott's Hill Farm, Bracknell, Berkshire, England (Ordinance Survey Reference SU 877738).
Pesticide use history at the collection site	None for the last 5 years.
Collection date	Not reported.
Collection procedures	Not reported.
Sampling depth (cm)	Depth of 10 cm after removing the top 5 cm of turf.
Storage conditions	Not reported.
Storage length	Not reported.
Soil preparation (eg: 2 mm sieved; air dried etc.)	Partially dried in a greenhouse at 25°C then sieved twice (5 mm mesh then 2 mm mesh).

Data obtained from p. 6 of MRID 41199102.

Table 2: Properties of the soils.

Property	East Jubilee (Soil Ref. No. 23/24)
Soil texture	Sandy loam
% Sand (0.05-2.0 mm)	62.6
% Silt (0.002-0.05 mm)	23.4
% Clay (<0.002 mm)	14
pH	6.5
Organic carbon (%)[1]	1.5
Organic matter (%)	2.6
CEC (meq/100 g)	13.2
Moisture at 15 bar (%)	7.4
Moisture at 1/3 bar (%)	12.9
Moisture at zero suction (%)	43.1
Maximum water holding capacity (%)	Not reported.
Bulk density (g/mL, disturbed)	Not reported.
Biomass (mg C/100 g soil)	39.7
Soil taxonomic classification	Not reported.
Soil mapping unit (for EPA)	Not reported.

Data obtained from Table 2, p. 16 of MRID 41199102.
1 Determined by the primary reviewer using the following equation: % organic carbon = % organic matter ÷ 1.724.

B. EXPERIMENTAL CONDITIONS:

1. Preliminary experiments: No preliminary experiments were described.

2. Experimental conditions:

Table 3: Experimental design.

Parameter		[^{14}C-Ring]BIT	[^{14}C-Carbonyl]BIT
Duration of the test (days)		180 days.	
Soil condition: (Air dried/fresh)		Partially dried at 25°C.	
Soil (g/replicate)		20 g (dry wt.).	
Application rates mg a.i./kg soil and equivalent g a.i./ha	Nominal	3 mg a.i./kg (56 mg/m^2; equivalent to 0.5 lb/acre, the predicted annual application rate). 100 mg a.i./kg (metabolite identification dose rate).	
	Actual	3 mg a.i./kg (test dose). 100 mg a.i./kg (high dose).	
Control conditions, if used		Sterile controls were not used.	
No. of Replications	Controls, if used	Sterile controls were not used.	
	Treatment	Duplicate.	

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Parameter		[^{14}C-Ring]BIT	[^{14}C-Carbonyl]BIT
Test apparatus	Type/material/ volume	The test system consisted of glass pots (38 mm diameter) containing moist treated soil (20 g dry wt) that were loaded into glass columns using stainless steel wire racks. The glass column was sealed at the top and bottom with septa containing air inlet and outlet tubes. Humidified, carbon dioxide-free air was passed through the column. The test apparatus was illustrated in Figure 2, p. 25.	
	Details of traps for CO_2 and organic volatiles, if any	Volatiles in the out-going air were trapped using tubes filled with the following sequential series of sorbents: glass wool; 0.1M HCl; 2-methoxyethanol; ethanolamine; ethanolamine; glass wool; 2M NaOH; and 0.05M H_2SO_4. The volatile trapping series was illustrated in Figure 2, p. 25.	
If no traps were used, is the system closed/open?		Closed.	
Identity and concentration of co-solvent		Aqueous sodium hydroxide, *ca.* 1% (200 µL added to 20 g dry weight).	
Test material	Volume of the test solution used/treatment:	200 µL (3 mg a.i./kg). 2 mg (100 mg a.i./kg; volume not speciifed).	
	Application method (*e.g.*: mixed/not mixed):	The test solution was added dropwise to the soil surface.	
	Is the co-solvent evaporated?	No.	
Any indication of the test material adsorbing to the walls of the test apparatus?		No.	
Microbial biomass/microbial population of the control (units)		Initial	Final
		Sterile controls were not used.	
Microbial biomass of the untreated soil (mg C_{bio}/ kg soil)		Initial	Final
		397	Not determined
Microbial biomass of the treated soil (mg C/100 g soil)		Initial	Final
		Not determined.	
Experimental conditions:	Temperature (°C):	20°C.	
	Continuous darkness (Yes/No):	Not reported.	
	Moisture content:	40% of moisture holding capacity (at zero suction)	
	Moisture maintenance method:	Samples were re-moistened with Milli-R0 water as necessary.	
Other details, if any		In some columns, sodium hydroxide was used in the place of ethanolamine to trap carbon dioxide.	

Data obtained from pp. 5-9; Table 1, pp. 15-16; Table 5, pp. 19-20; Figure 2, p. 25 of MRID 41199102.

3. Aerobic conditions: Aerobic conditions were maintained by passing humidified, carbon dioxide-free air through the column (p. 8; Figure 2, p. 25 of MRID 41199102). No determinations were made to verify that aerobic conditions were maintained.

4. Supplementary experiments: None reported.

5. Sampling:

Table 4: Sampling details.

Criteria	[^{14}C-Ring]BIT	[^{14}C-Carbonyl]BIT
Sampling intervals (days posttreatment)	3 mg/kg: time 0 and 1, 4, 7, 14, 28, 60, 98, 125, 161 and 180 days posttreatment. 100 mg/kg: time 0 and 7, 14, 28, 98 and 180 days posttreatment.	
Sampling method	Duplicate flasks collected at each interval.	
Method of collection of CO_2 and organic volatile compounds	Volatiles were collected at 4, 7, 14, 28, 60, 98, 125, 161 and 180 days posttreatment.	
Sampling intervals/times for: Sterility check, if sterile controls are used: Moisture content: Redox potential, other:	 Sterile controls were not used. Moisture was adjusted at sampling times or monthly. None determined.	
Sample storage before analysis	Samples were analyzed immediately; no sample storage was reported prior to initial analysis. Extracts were stored frozen (temperature not reported) prior to metabolite identification using selected high-dose samples.	
Other observation, if any	None reported.	

Data obtained from p. 9; Table 1, p. 15; Tables 5-6, pp. 19-21; Table 8, p. 23 of MRID 41199102.

C. ANALYTICAL METHODS:

Extraction/clean up/concentration methods: Soil samples were extracted on a cellulose thimble in a Soxhlet extraction apparatus with 100 mL of acetonitrile for 2 hours then with 90 mL of acetonitrile:1M HCl (80:20, v:v) for 2 hours (p. 9 of MRID 41199102). The final volume of each extract was adjusted to 100 mL using acetonitrile prior to analysis via LSC and TLC. Extracts were stored frozen after use. The sample processing procedure was illustrated in Table 4, p. 18 of MRID 41199102.

Total ^{14}C measurement: Total residues were determined as the sum of the radioactivity measured in the soils extracts, volatile traps and nonextractable residues (Table 5, pp. 19-20 of MRID 41199102).

Determination of nonextractable residues: After extraction, the soil residue and cellulose thimble were air-dried and finely ground; a subsample was analyzed for total radioactivity using LSC/combustion (p. 10 of MRID 41199102). Combustion efficiency was >88%.

Determination of volatile residues: Volatiles in tubes containing 0.1M HCl, 2-methoxyethanol, ethanolamine and 2M NaOH were measured via LSC (pp. 8-9 of MRID 41199102). The radioactivity in the NaOH traps was characterized as carbon dioxide using $BaCl_2$ precipitation prior to LSC analysis.

Derivatization method, if used: None was reported.

Identification and quantification of parent compound: Soil extracts were analyzed for the parent using normal- and reversed-phase TLC analysis (pp. 10-11 of MRID 41199102). Normal-phase TLC was performed using either Camlab SIL C-25/UV254 precoated plates or Whatman LK6F silica gel precoated plates developed with the following solvent system: toluene:ethyl acetate:glacial acetic acid:water:ethanol (80:10:0.5:0.5:9, v:v:v:v:v). For purity checks, the following solvent system was used: cyclohexane:ethanol (80:20, v:v). Reversed-phase TLC was performed using either Whatman KC18F or Whatman LKC18F precoated plates developed with methanol:water (80:20, v:v). The parent, BIT, was identified via co-chromatography with the unlabeled BIT reference sample (1,2-benzisothiazolin-3-one; chemical purity 99.7%; Brixham Reference No. R255; pp. 7, 11 of MRID 41199102). Radiolabeled and unlabeled parent was visualized using UV (254 nm). The parent was quantified using a Raytest RITA 6800 Automatic TLC Linear Analyzer. For further quantification, a HPLC method was employed using a Spherisorb S50DS2 column (25 cm x 4.6 mm) and a mobile phase of Milli-R0 water:acetonitrile:methanol:acetic acid (73:14:10:3, v:v:v:v) with a flow rate of 1.5 mL/min (pp. 11-12 of MRID 41199102).

In a subsequent analysis, further confirmation of the identification of the parent was performed with the selected high-dose (100 mg/kg) extracts which contained >10% of the applied radioactivity (day 7 ring-labeled and day 28 both labels) using TLC analyses (HPLC and MS analyses were only used for transformation products; pp. 8-10 of MRID 41199103). Prior to those analytical identification methods, the duplicate extracts were combined, concentrated and analyzed by LSC to quantify total radioactivity. Normal-phase TLC was performed using 60A LK6F/UV_{254} silica gel precoated plates developed with the following solvent systems: (a) toluene:ethyl acetate:glacial acetic acid:water:ethanol (80:10:0.5:0.5:9, v:v:v:v:v) or (b) chloroform:methanol:glacial acetic acid (65:35:5, v:v:v). Reversed-phase TLC was performed using Whatman LKC 18F linear-k plates developed with methanol:water (80:20, v:v). The parent was identified via co-chromatography and comparison of R_f values with the unlabeled BIT reference sample (1,2-benzisothiazolin-3-one; chemical purity not reported; Batch No.: ODAS 129; p. 10; Figure 1, p. 20 of MRID 41199103). Radiolabeled and unlabeled parent was visualized using UV (200 nm or 254 nm). The parent was quantified using an Isomess Raytest RITA 68000 TLC Linear Analyzer or a Berthold LB2842 automatic TLC linear analyzer (p. 11 of MRID 41199103).

Identification and quantification of transformation products: Transformation products were identified and quantified using the same methods as described for the parent (pp. 10-12 of MRID 41199102).

Selected high-dose (100 mg/kg) extracts which contained >10% of the applied radioactivity (day 7 ring-labeled and day 28 both labels) were subjected to the same subsequent further TLC analysis for

compound identification as described above for the parent (p. 8; Table 1, p. 46 of MRID 41199103). The following reference standards were included for identification of the transformation products: BIT-S-oxide (1,2-benzisothiazolin-3-one-1-oxide; Batch No.: GLP 911, ODAS 078; chemical purity not reported); saccharin (1,2-benzisothiazolin-3-one-1,1-dioxide; Batch No.: GLP 913; chemical purity not reported); and o-sulphobenzamide (2-carbamoylbenzenesulphonic acid; Batch No.: GLP 924; chemical purity not reported; Figure 1, p. 20 of MRID 41199103). Unknowns in the high-dose (100 mg/kg) extracts were also identified using the following HPLC method: a Spherisorb S50DS2 column (25 cm x 4.6 mm) eluted at 1 mL/min with A:B (20:80, v:v) where (A) acetonitrile:methanol:glacial acetic acid:distilled water (14:10:3:73, v:v:v:v) buffered to pH 4.0 with ammonia and (B) distilled water (p. 12 of MRID 41199103). Identifications were made using UV (254 nm) and radiodetection. HPLC/MS analysis was also performed using that HPLC column and eluent coupled with a Finnigan 4500 mass spectrometer (pp. 13-14 of MRID 41199103).

Detection limits (LOD, LOQ) for the parent compound: The Limits of Quantification (LOQ) and the Limit of Detection (LOD) were not reported.

Detection limits (LOD, LOQ) for the transformation products: The Limits of Quantification (LOQ) and the Limit of Detection (LOD) were not reported.

II. RESULTS AND DISCUSSION

A. TEST CONDITIONS: No supporting records were provided to establish that aerobicity was maintained throughout the 180 day study. The temperature was 20°C (range not provided; p. 5 of MRID 41199102). Soil moisture was 40% of maximum water holding capacity at zero suction (p. 8 of MRID 41199102). Initial microbial biomass was determined to be 397 mg C_{bio}/kg soil; no further measurements were reported (Table 2, p. 16 of MRID 41199102). .

B. MATERIAL BALANCE: For the 3 mg/kg dose rate on East Jubilee sandy loam soil, the overall recovery was 99.13 ± 1.82% (mean, range 94.74-102.41%) of the applied radioactivity for the [^{14}C-ring]BIT and 91.67 ± 10.27% (mean, range 81.96-103.06% with one outlier at time 0 of 69.94%) for the [^{14}C-carbonyl]BIT (Table 5, pp. 19-20 of MRID 41199102). For the 100 mg/kg dose rate on East Jubilee sandy loam soil, the overall recovery was 95.99 ± 3.46% (mean, range 91.36-102.73%) for the [^{14}C-ring]BIT and 97.47 ± 3.07% (mean, range 91.92-101.61%) for the [^{14}C-carbonyl]BIT (Table 5, pp. 19-20 of MRID 41199102). There was a noticeable pattern of decline for all experiments, except the 3 mg/kg dose with [^{14}C-ring]BIT.

Table 5a: Biotransformation of [^{14}C-ring]1,2-benzisothiazolin-3-one (BIT), expressed as percentage of applied radioactivity, applied at 3 mg/kg to East Jubilee sandy loam soil from England under aerobic conditions.

Compound	Sampling Intervals (days)									
	0	**1**	**4**	**7**	**14**	**28**	**60**	**98**	**125**	**180**
BIT	--[1]	--	--	--	--	--	--	--	--	--
Other [^{14}C]residues	--	--	--	--	--	--	--	--	--	--
Extractable residues[2]	88.96 ± 3.63	41.86 ± 1.95	37.14 ± 7.16	19.61 ± 7.04	24.55 ± 15.53	29.86 ± 1.87	6.13 ± 1.81	6.11 ± 2.24	5.41 ± 0.37	2.60 ± 0.63
Nonextractable residues	11.82 ± 3.80	54.02 ± 0.35	60.05 ± 6.72	72.98 ± 6.52	65.39 ± 17.18	55.72 ± 2.83	59.80 ± 3.20	56.30± 2.52	54.29 ± 1.03	54.73 ± 0.95
CO_2	NM	NM	3.23[3]	5.23	8.33	16.15	32.07	36.88	39.22	42.95
Volatile organics	Insignificant radioactivity.									
Total Recovery	100.78 ± 0.18	95.88 ± 1.61	100.41 ± 0.44	97.82 ± 0.52	98.27 ± 1.65	101.73 ± 0.96	98.00 ± 1.39	99.29 ± 0.28	98.91 ± 0.65	100.28 ± 0.32

Data obtained from p. 12; Table 5, p. 19 of MRID 41199102. Means and standard deviations were calculated by the primary reviewer.
1 Not determined in MRID 41199102 or MRID 41199103.
2 The sum of the radioactivity found in the acetonitrile and acetonitrile:HCl extracts.
3 Single values reported for carbon dioxide measurements at each sampling interval.
NM - Not measured.

Table 5b: Biotransformation of [^{14}C-carbonyl]1,2-benzisothiazolin-3-one (BIT), expressed as percentage of applied radioactivity, applied at 3 mg/kg to East Jubilee sandy loam soil from England under aerobic conditions.

Compound	Sampling Intervals (days)									
	0	1	4	7	14	28	60	98	125	180
BIT	--[1]	--	--	--	--	--	--	--	--	--
Other [^{14}C]residues	--	--	--	--	--	--	--	--	--	--
Extractable residues[2]	67.80 ± 14.22	38.44 ± 8.49	38.51 ± 8.24	35.87 ± 8.22	28.07 ± 8.99	10.59 ± 0.71	4.09 ± 1.00	3.83 ± 0.36	3.13 ± 0.35	2.34 ± 0.43
Nonextractable residues	18.17 ± 8.44	62.44 ± 9.26	60.14 ± 6.47	59.12 ± 5.56	62.00 ± 9.30	70.60 ± 1.75	48.11 ± 1.34	45.65 ± 0.45	44.42 ± 0.30	42.99 ± 0.04
CO_2	NM	NM	3.17[3]	5.74	9.92	16.06	30.29	33.30	34.88	37.12
Volatile organics	Insignificant radioactivity.									
Total Recovery	85.97 ± 22.66	100.88 ± 0.77	101.81 ± 1.77	100.73 ± 2.65	99.99 ± 0.30	97..25 ± 2.46	82.49 ± 0.35	82.78 ± 0.09	82.42± 0.65	82.45 ± 0.47

Data obtained from p. 12; Table 5, p. 20 of MRID 41199102. Means and standard deviations were calculated by the primary reviewer.
1 Not determined in MRID 41199102 or MRID 41199103.
2 The sum of the radioactivity found in the acetonitrile and acetonitrile:HCl extracts.
3 Single values reported for carbon dioxide measurements at each sampling interval.
NM - Not measured.

Table 5c: Biotransformation of [^{14}C-ring]1,2-benzisothiazolin-3-one (BIT), expressed as percentage of applied radioactivity, applied at 100 mg/kg to East Jubilee sandy loam soil from England under aerobic conditions.

Compound	Sampling Intervals (days)					
	0	**7**	**14**	**28**	**98**	**180**
BIT	--[1]	--	--	--	--	--
o-Sulphobenzamide	ND	14.16	ND	26.47	ND	ND
Saccharin	ND	5.94	ND	4.32	ND	ND
Unknown	ND	0.00	ND	1.24	ND	ND
BIT-S-oxide	ND	23.79	ND	1.89	ND	ND
Extractable residues[2]	96.47 ± 4.19	51.10 ± 9.89	37.07 ± 14.08	43.63 ± 5.55	6.70 ± 2.21	3.78 ± 0.40
Nonextractable residues	3.09 ± 0.30	45.62 ± 11.33	58.47 ± 16.53	48.61 ± 4.90	44.73 ± 1.34	44.00 ± 0.08
CO_2	NM	1.42[3]	2.42	3.75	41.29	43.81
Volatile organics	Insignificant radioactivity.					
Total Recovery	99.56 ± 4.49	98.14 ± 1.44	97.95 ± 2.45	95.98 ± 0.65	92.72 ± 0.87	91.59 ± 0.32

Data obtained from p. 12; Table 5, p. 19 of MRID 41199102 and Table 3, p. 48 of MRID 41199103. Means and standard deviations were calculated by the primary reviewer.

1 Not determined in MRID 41199102 or MRID 41199103.

2 The sum of the radioactivity found in the acetonitrile and acetonitrile:HCl extracts.

3 Single values reported for carbon dioxide measurements at each sampling interval.

ND - Not determined, only selected samples were analyzed for [^{14}C]residue identification.

NM - Not measured.

Table 5d: Biotransformation of [^{14}C-carbonyl]1,2-benzisothiazolin-3-one (BIT), expressed as percentage of applied radioactivity, applied at 100 mg/kg to East Jubilee sandy loam soil from England under aerobic conditions.

Compound	Sampling Intervals (days)					
	0	7	14	28	98	180
BIT	--[1]	--	--	--	--	--
o-Sulphobenzamide	ND	ND	ND	28.46	ND	ND
Saccharin	ND	ND	ND	4.32	ND	ND
Unknown	ND	ND	ND	1.73	ND	ND
BIT-S-oxide	ND	ND	ND	1.72	ND	ND
Extractable residues[2]	94.25 ± 2.84	53.80 ± 1.97	37.24 ± 9.79	39.97 ± 3.42	3.38 ± 0.99	2.90 ± 0.04
Nonextractable residues	5.03 ± 1.69	45.11 ± 0.47	54.87 ± 12.64	49.33 ± 4.91	37.68 ± 1.21	34.65 ± 0.94
CO_2	NM	0.84[3]	3.87	11.26	55.62	55.07
Volatile organics	Insignificant radioactivity.					
Total Recovery	99.27 ± 1.15	99.75 ± 1.49	95.97 ± 2.84	100.56 ± 1.49	96.68 ± 0.22	92.62 ± 0.98

Data obtained from p. 12; Table 5, p. 20 of MRID 41199102 and Table 3, p. 48 of MRID 41199103. Means and standard deviations were calculated by the primary reviewer.

1 Not determined in MRID 41199102 or MRID 41199103.

2 The sum of the radioactivity found in the acetonitrile and acetonitrile:HCl extracts.

3 Single values reported for carbon dioxide measurements at each sampling interval.

ND - Not determined, only selected samples were analyzed for [^{14}C]residue identification.

NM - Not measured.

C. TRANSFORMATION OF PARENT COMPOUND: The amounts of the parent, 1,2-benzisothiazolin-3-one (BIT), were not determined throughout the study (MRID 41199102) or the supporting study (MRID 41199103). The study author reported that "only trace amounts" of the parent were present in the soil extracts sampled at 1 and 4 days posttreatment (p. 5 of MRID 41199102).

HALF-LIFE/DT50/DT90: The half-lives ranged from 5.6-12.2 days for the anticipated application rate (3 mg/kg) and 16-17 days for the exaggerated rate (100 mg/kg) that was intended to generate any degradates that may form. The observed half-lives were 1-7 days for the 3 mg/kg rate. There were no significant degradates that occurred on a consistent basis, although two were present sporadically. These were ortho-sulphobenzamide and BIT-S-oxide.

Half-lives/DT50/DT90

Compound	Half-life/DT50 (days)	First order linear regression equation	r^2	DT90 (days)
Linear/natural log	42	36.2*exp(-0.016*time)	0.79	140
	38	37.2*exp(-0.018*time)	0.81	125
Nonlinear/normal	5.6	68.76*EXP(-0.12*time)	0.67	18.6
	12.2	53.9*EXP(-0.057*time)	0.86	41
Observed DT50	1-7	None	None	28-60 7-14

TRANSFORMATION PRODUCTS: Transformation products were not determined in the initial study (MRID 41199102); however, selected high dose samples were analyzed for transformation products in a follow-up study (MRID 41199103). o-Sulphobenzamide (2-carbamoylbenzenesulphonic acid) was observed as a major transformation product in all samples (day 7 ring-labeled and day 28 both labels) with maximums of 26.47% and 28.46% of the applied radioactivity at 28 days for the [^{14}C-ring]BIT and [^{14}C-carbonyl]BIT, respectively (14.16% at 7 days for the [^{14}C-ring]BIT; Table 3, p. 48 of MRID 41199103). BIT-S-oxide (1,2-benzisothiazolin-3-one-1-oxide) was observed as a major transformation product in the [^{14}C-ring]BIT samples at 7 days (23.79%) and a minor transformation product at 28 days (1.89% and 1.72% for the [^{14}C-ring]BIT and [^{14}C-carbonyl]BIT, respectively). Saccharin (1,2-benzisothiazolin-3-one-1,1-dioxide) was observed as a minor transformation product in all samples with maximums of 5.94% at 7 days for the [^{14}C-ring]BIT and 4.32% at 28 days for the [^{14}C-carbonyl]BIT. Unknown radioactivity was measured at maximums of 1.24% and 1.73% at 28 days for the [^{14}C-ring]BIT and [^{14}C-carbonyl]BIT, respectively.

Table 6: Chemical names and CAS numbers for the transformation products of 1,2-benzisothiazolin-3-one (BIT).

Applicants Code Name	CAS Number	Chemical Name	Chemical Formula	MW (g/mol)	Smiles String
BIT-S-oxide		1,2-Benzisothiazolin-3-one-1-oxide.			
o-Sulphobenzamide		2-Carbamoylbenzenesulphonic acid.			
Saccharin		1,2-Benzisothiazolin-3-one-1,1-dioxide.			

Data obtained from Figure 1, p. 20 of MRID 41199103.

NONEXTRACTABLE AND EXTRACTABLE RESIDUES: For the 3 mg/kg treatment of [^{14}C-ring]BIT on East Jubilee sandy loam soil, the total extractable residues declined rapidly from the maximum of 88.96% of the applied radioactivity at time 0 to 41.86% at 1 day to 19.61-29.86% at 7-28 days to 6.13% at 60 days and was 2.60% at 180 days (study termination; Table 5, pp. 19-20 of MRID 41199102). Corresponding nonextractable residues increased from 11.82% at time 0 to a maximum of 72.98% at 7 days then declined to 54.29-54.73% at 125-180 days. For the [^{14}C-carbonyl]BIT treatment, the total extractable residues declined rapidly from the maximum of 67.80% at time 0 to 38.44% at 1 day to 10.59% at 28 days to 4.09% at 60 days and was 2.34% at 180 days (study termination). Corresponding nonextractable residues increased from 18.17% at time 0 to a maximum of 70.60% at 28 days then declined to 42.99% at 180 days.

For the 100 mg/kg treatment of [^{14}C-ring]BIT on East Jubilee sandy loam soil, the total extractable residues declined rapidly from the maximum of 96.47% of the applied radioactivity at time 0 to 51.10% at 7 days to 37.07-43.63% at 14-28 days to 6.70% at 98 days and was 3.78% at 180 days (study termination; Table 5, pp. 19-20 of MRID 41199102). Corresponding nonextractable residues increased from 3.09% at time 0 to a maximum of 58.47% at 14 days then declined to 44.00% at 180 days. For the [^{14}C-carbonyl]BIT treatment, the total extractable residues declined rapidly from the maximum of 94.25% at time 0 to 53.80% at 7 days to 37.24-39.97% at 14-28 days to 3.38% at 98 days and was 2.90% at 180 days (study termination). Corresponding nonextractable residues increased from 5.03% at time 0 to a maximum of 54.87% at 14 days then declined to 34.65% at 180 days.

VOLATILIZATION: At 180 days (study termination), $^{14}CO_2$ was measured at maximums of 42.95% and 37.12% of the applied radioactivity for the [^{14}C-ring]BIT and [^{14}C-carbonyl]BIT, respectively, applied to East Jubilee sandy loam soil at 3 mg/kg (Table 5, pp. 19-20 of MRID 41199102). For the 100 mg/kg treatment, $^{14}CO_2$ was measured at maximums of 43.81% (180 days, study termination) for the [^{14}C-ring]BIT and 55.62% (98 days; 55.07% at 180 days, study termination) for the [^{14}C-carbonyl]BIT. Other organic volatiles were observed in insignificant quantities in all experiments (p. 12 of MRID 41199102).

TRANSFORMATION PATHWAY: The study author of MRID 41199103 described a transformation pathway for 1,2-benzisothiazolin-3-one (BIT; pp. 7, 19 of MRID 41199103). BIT-S-oxide (1,2-benzisothiazolin-3-one-1-oxide) and saccharin (1,2-benzisothiazolin-3-one-1,1-dioxide) were formed by the sequential oxidation of the parent, BIT. o-Sulphobenzamide (2-carbamoylbenzenesulphonic acid) was formed by ring hydrolysis of saccharin. Extensive

mineralization to carbon dioxide was observed (p. 5 of MRID 41199102). Additionally, a large amount of bound residues were observed.

D. SUPPLEMENTARY EXPERIMENT-RESULTS: No supplemental experiments were reported.

III. STUDY DEFICIENCIES

None

IV. REVIEWER'S COMMENTS

1. The two studies were submitted as accompanying studies, where the experimental was performed in the first study (MRID 41199102) and the characterization of non-volatile [^{14}C]residues was performed in the second study (MRID 41199103). The primary reviewer did not separate the studies in the DER.

2. The radioactive purity was low (91.7%) for the [^{14}C-ring]BIT (Table 3, p. 17 of MRID 41199102).

V. REFERENCES

1. U.S. Environmental Protection Agency. 1982. Pesticide Assessment Guidelines, Subdivision N, Chemistry: Environmental Fate, Section 162-1, Aerobic Soil Metabolism Studies. Office of Pesticide and Toxic Substances, Washington, DC. EPA 540/9-82-021.

2. U.S. Environmental Protection Agency. 1989. FIFRA Accelerated Reregistration, Phase 3 Technical Guidance. Office of the Prevention, Pesticides, and Toxic Substances, Washington, DC. EPA 540/09-90-078.

3. U.S. Environmental Protection Agency. 1993. Pesticide Registration Rejection Rate Analysis - Environmental Fate. Office of the Prevention, Pesticides, and Toxic Substances, Washington, DC. EPA 738-R-93-010.

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